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CHD8, A Novel Beta-Catenin Associated Chromatin Remodeling Enzyme, Regulates Androgen Receptor Mediated Gene Transcription

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14. ABSTRACT

The activity of the androgen receptor (AR) is critical for normal prostate development and function, but AR activity also plays a major role in the development and progression of prostate cancer. To better understand the function of beta-catenin in AR mediated transcription, we have identified a novel chromatin remodeling enzyme, CHD8, that can associate with beta-catenin and functions in AR mediated gene transcription. Year 2 was focused on the continuing the first specific aim of this proposal (the interaction of CHD8 with beta-catenin and the androgen receptor) and starting the second specific aim (the function of CHD8 and beta-catenin at the PSA promoter). This work is required for the study of the function of CHD8 and beta-catenin in AR mediated transcription and the ultimate understanding of the role played by CHD8 in prostate cancer progression.

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TABLE OF CONTENTS

INTRODUCTION

The activity of the androgen receptor (AR) is critical for normal prostate development and function, but AR activity also plays a major role in the development and progression of prostate cancer. Current therapies utilize this fact and are aimed at reducing serum androgens and therefore, inhibiting AR. While these therapies show initial success in preventing tumor growth, they can ultimately fail due to the development of an androgen independent disease. β-catenin, a known oncogene, has been shown to directly interact with AR and also to function as a transcriptional coactivator for AR. To better understand the function of β-catenin in AR mediated transcription, we have identified a novel chromatin remodeling enzyme, CHD8, that can associate with β-catenin and functions in AR mediated gene transcription. The identification and characterization of novel factors, such as CHD8, that can function as coactivators of AR mediated transcription could result in the discovery of novel diagnostic, preventative, or therapeutic targets for prostate cancer.

BODY

β-catenin, a known oncogene, functions as a transcriptional coactivator for AR through a direct protein/protein interaction (1-5). Although the significance of this interaction is not known, understanding the crosstalk between these oncogenic pathways is critical to delimitating the function of AR in tumorigenesis. To better understand the function of β-catenin in AR mediated transcription, we have identified novel chromatin remodeling enzyme, CHD8 that can associate with β-catenin and functions in AR mediated gene transcription. **The hypothesis of this proposal is that through a direct interaction with β-catenin, CHD8 is directed to androgen responsive promoters and functions in regulating transcription by altering the chromatin structure.** This hypothesis will be tested by addressing the following Aims: 1) Does β-catenin target CHD8 to AR responsive promoters? 2) Are CHD8 and β-catenin both required to affect AR transcriptional activity? 3) Is CHD8 required for prostate cancer progression? The proposed studies will provide detailed information concerning the role of β-catenin and CHD8 in AR mediated transcription. This information will be valuable for the understanding of how β-catenin mediates AR transcriptional activation, and also will provide general insight into the mechanisms of prostate neoplastic transformation through the control of chromatin structure.

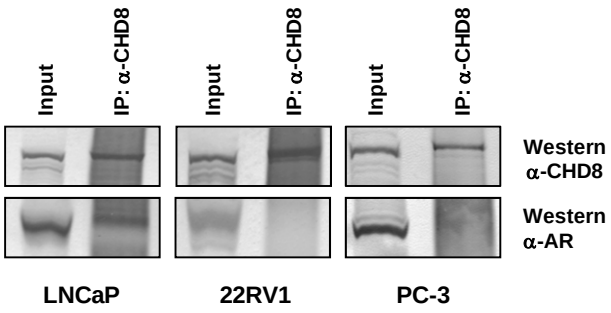

Figure 1. CHD8 interacts with AR in LNCaP, but not 22RV1 or PC-3 cell lines. Nuclear extracts were prepared from the indicated cell lines and incubated with CHD8 antibodies. Following precipitation with Protein A agarose and extensive washing, purified samples were subjected to SDS-PAGE followed by Western blotting analysis using the antibodies indicated in the figure.

The majority of the work proposed in months 12-24 addresses Specific Aim 2: Are CHD8 and β-catenin both required to

affect AR transcriptional activity? As outlined in the 2008 Annual Report, several obstacles were encountered that required alternative approaches or additional effort. Therefore, this report outlines progress in several Tasks from Year 1 that were required for the continuation of Tasks planned for Year 2. Upon completion of these Tasks, progress was made on the majority of Tasks planned for Year 2. These advances have been sufficient to allow for the start of Tasks planned for Year 3.

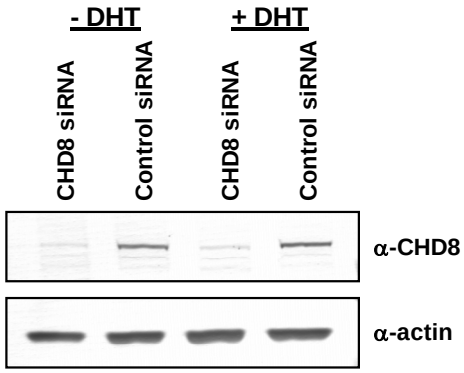

Figure 2. Depletion of endogenous CHD8 in LNCaP cells. LNCaP cells were transfected with control shRNA or CHD8 shRNA. Following selection of the transfected cells, extracts were prepared and subjected to SDS-PAGE/Western blot analysis with CHD8 antibodies. Actin is blotted as a loading control.

Investigations listed in the 2008 Annual Report demonstrated a direct interaction between CHD8 and AR *in vitro* (Task 1a-b). Further proposed studies will then address the *in vivo* association of these proteins at endogenous promoters (Task 1d). Prior to completing this Task, a verification of the association of CHD8 and AR was performed *in vivo*. For this experiment three cell lines were used, LNCaP, 22RV1 and PC-3. LNCaP cells are an androgen dependent cell line and will be used in Tasks 1c-2c. 22RV1 and PC-3 cells are androgen independent cell lines and were included in this study for comparison. Nuclear extracts were prepared and samples were then immunoprecipitated with antibodies directed against CHD8. After extensive washing, immunoprecipitated samples were subjected to SDS-PAGE followed by Western blot analysis with antibodies against CHD8 or AR (Figure 1). In agreement with the *in vitro* interaction data, CHD8 interacts with AR in the androgen dependent cell line, LNCaP. This result confirms their association and demonstrates that this cell line is a suitable choice for the completion of Aims 1 and 2. It was interesting to also note that CHD8 only interacted with AR in androgen dependent cell lines, although AR was present in all cell lines tested (Figure 1). This result further strengthens the argument for Experiment 3.1 that addresses the requirement for CHD8 in progression from androgen dependent to androgen independent growth.

Experiments outlined in all three Tasks require the creation of stable cell lines that express shRNA to CHD8 and β-catenin (Task 1c, 2a, 3a). Various difficulties were encountered in Year 1, and these difficulties have been overcome allowing the creation of LNCaP cell lines with shRNA targeting CHD8 (Figure 2). Importantly, the reduction of CHD8 is independent of the presence of the AR ligand dihydrotestosterone (DHT). This cell line can now be used for the remainder of the Tasks outlined in 1 and 2. These cells also provide a viable alternative to the use of VCaP cells outlined in Task 3 should difficulties be encountered creating that cell line.

The creation of the LNCaP stable cell lines in Figure 2 has allowed for the start of the experiments outlined in Task 2b to test the requirement of CHD8 in AR mediated transcription. Two endogenous androgen responsive targets were chosen for analysis, PSA and TMPRSS2.

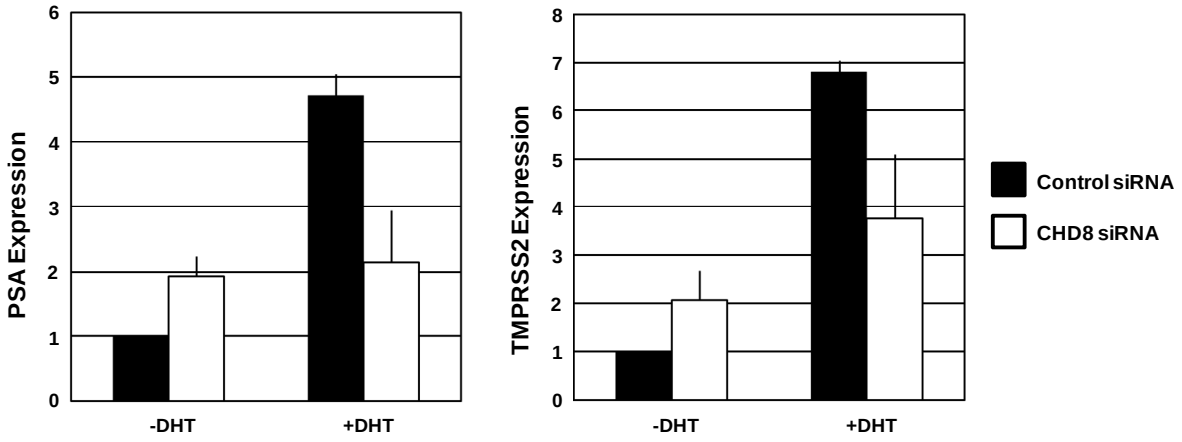

Figure 3. CHD8 regulates AR target genes. LNCaP cells were transfected with control shRNA or CHD8 shRNA. Following selection of the transfected cells, total RNA was harvested and expression of the indicated genes was analyzed by real-time RT-PCR. Shown is a typical result from multiple experiments.

Analysis of the PSA gene was outlined in the original proposal, and TMPRSS2 is a newly identified androgen responsive gene that is fused to the ETS transcription factor in most prostrate cancers (6,7). The use of TMPRSS2 will further strengthen the results of these studies. LNCaP cells with shRNA to CHD8 or a control shRNA were treated with DHT or vehicle. cDNA was then isolated and analyzed by qPCR with primers to PSA or TMPRSS2 (Figure 3). Consistent with the original hypothesis, upon induction with DHT depletion of CHD8 results in an approximate 2 fold decrease in the expression of both PSA and TMPRSS2. Of interest is that depletion of CHD8 results in an up regulation of these targets in the absence of DHT, and this up regulation is comparable to level of expression without CHD8 in the presence of DHT. These results suggest that these genes have now lost their ability to be regulated by AR. This is not due to the loss of AR expression as western blot analysis shows no change in AR protein levels upon depletion of CHD8 (data not shown).

The direct interaction of CHD8 with AR both *in vitro* and *in vivo*, and the fact that CHD8 is required for the DHT stimulated transcription of androgen target genes, further supports the hypothesis that CHD8 and AR interact at target genes to regulate androgen dependent transcription. The first step in testing this hypothesis is to demonstrate that CHD8 and AR can both bind to androgen responsive elements (AREs) at target genes. To test this, chromatin immunoprecipitation (ChIP) experiments were performed (Task1d). These studies were performed on AREs from both the PSA and TMPRSS2 genes in LNCaP cells treated with DHT. Figure 4 demonstrates that CHD8 and AR are both bound to these promoters and the availability of the LNCaP stable cell lines in Figure 2 will allow for the continuation of this Task (Task1d). In addition, two histone methylation marks were also tested for analysis in Task 2c.

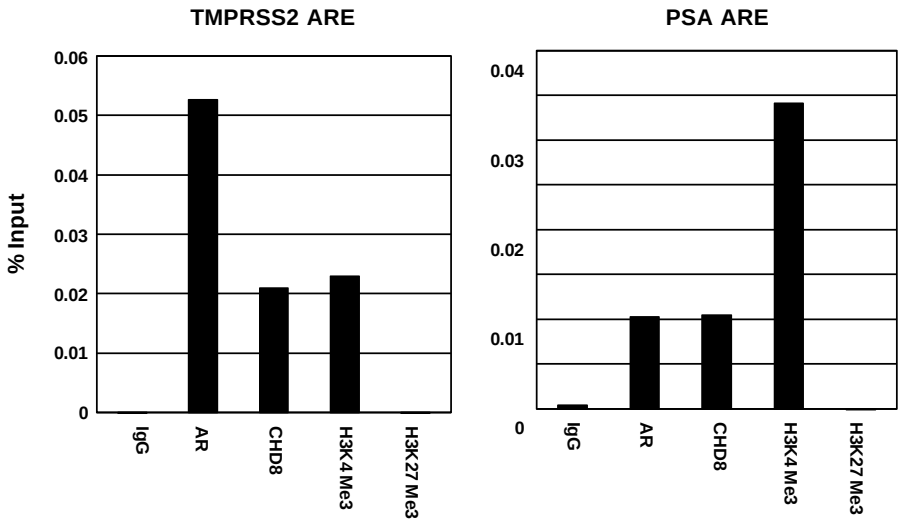

Figure 4. CHD8 is bound to β catenin responsive promoters. Chromatin from DHT treated LNCaP prostate cancer cells was cross linked *in vivo* with formaldehyde. Cells were lysed, and chromatin immunoprecipitations were performed with the indicated antibody. Immunoprecipitates were extensively washed and the cross linking was reversed. Bound DNA was detected by quantitative PCR with primer pairs to the indicated ARE.

KEY RESEARCH ACCOMPLISHMENTS

- Protein interaction studies have been performed to demonstrate the interaction of CHD8 and AR *in vivo*. This result is a continuation of Task 1b.
- Stable LNCaP cell lines have been made for the *in vivo* knockdown of CHD8 (Task 1c and Task 2a).
- Chromatin immunoprecipitation experiments were commenced to detect the association of AR and CHD8 at androgen responsive promoters (Task 1d). These assays also included various antibodies directed against histone methylation marks (Task 2c)
- A direct role for CHD8 in AR mediated transcription has been demonstrated using CHD8 knockdown LNCaP cells (Task 2b).
- Although a stable VCaP cell line has yet to be created (Task 3a), the creation of a stable knockdown of CHD8 in LNCaP provides a workable alternative for the experiments outlined in Year 3 (Task 3b and 3c).

REPORTABLE OUTCOMES

The data presented here has been presented locally at the University of Michigan, both in the department, as well as interdepartmentally. In addition, portions of these data have also been presented at the ASBMB Transcriptional Regulation by Chromatin and RNA Polymerase II meeting in Lake Tahoe, CA, and also at the Michigan State University Summer Symposium on Transcriptional Regulation and Systems Biology meeting in East Lansing, MI.

CONCLUSION

As outlined in the original proposal, the majority of work proposed for Year 2 was centered on the role of CHD8 and β-catenin in AR mediated gene transcription *in vivo* at endogenous

promoters. Many of these tasks have been addressed along with the continuation of tasks from Year 1 that required alternative approaches. These studies are on track for the investigation of the role played by CHD8 in prostate cancer progression (Year 3). This understanding can lead to the development of chromatin remodeling inhibitors as therapeutics in prostate cancer.

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APPENDICES

None